From Cell Membrane to Nucleus: The Effects of Alcohol on Brain Neurons

Alcohol alters synaptic transmission; a great deal of research over many years has suggested that this is the major change in the brain that gives rise to intoxication. As described in the first section of this chapter, "Setting the Stage: The Structure and Function of Neurons," a number of neurotransmitters are involved in synaptic communication within the brain. Alcohol affects the functions of several of these neurotransmitters by altering the communication between neurons that occurs when the neurotransmitter activates its receptor.

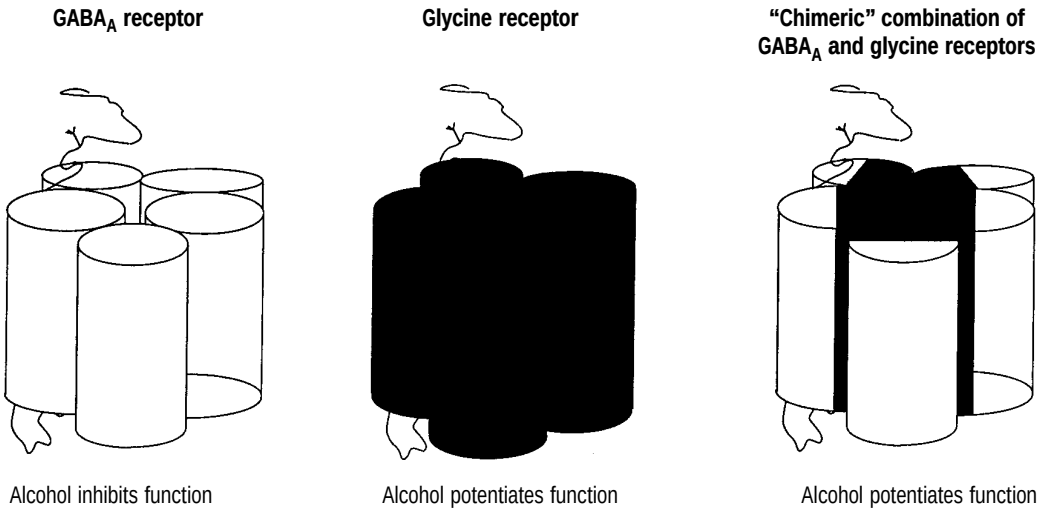
One of the most powerful effects of alcohol is to reduce the pace of brain activity by a combination of effects that reduces the excitatory actions of the neurotransmitter glutamate and enhances the inhibitory actions of the neurotransmitters gamma-aminobutyric acid (GABA) and glycine (Diamond and Gordon 1997; Korpi 1994; Lovinger 1997; Machu 1996; Mascia et al. 1996; Mhatre and Ticku 1993; Mihic et al. 1997; Sanna and Harris 1993). These actions are the main reason that alcohol is often thought of as a depressant.

Alcohol's Effect on Synaptic Transmission During Acute Exposure

The effects of alcohol on excitatory glutaminergic and inhibitory GABAergic and glycinergic synaptic transmission mainly result from alcohol's actions on the ligand-gated ion channels activated by these neurotransmitters. (See the section "Setting the Stage: The Structure and Function of Neurons" earlier in this chapter for background on the processes discussed here.) At synapses that use glutamate, alcohol reduces the activity of the neurotransmitter at ligand-gated ion channel receptors called the *N*-methyl-D-aspartate, or NMDA, class of glutamate receptors (Diamond and Gordon 1997; Lovinger 1996; Tabakoff and Hoffman 1995). Ion flow through the channel that is part of this receptor is reduced. This effect of alcohol may contribute to the memory loss that occurs during acute alcohol exposure, as will be discussed later in this section.

In contrast, alcohol enhances the activity of the inhibitory neurotransmitters GABA and glycine at receptors called $GABA_A$ and glycine ligand-gated ion channels (Aguayo et al. 1996; Celentano et al. 1988; Diamond and Gordon 1997; Korpi 1994; Machu 1996; Mascia et al. 1996; Mhatre and Ticku 1993; Mihic et al. 1997; Sanna and Harris 1993). Exciting new information about the molecular actions of alcohol has come from studies of these receptors. Prior research demonstrated alcohol's opposite actions on two receptors that were similar in molecular structure, the $GABA_A$ receptor (subtype rho [ρ]) and the receptor glycine (subtype α_1) (Mihic and Harris 1996; Mihic et al. 1997). This finding provided an interesting opportunity for investigators to "swap" pieces of the receptors by using genetic recombination techniques (figure 1). By cutting pieces of deoxyribonucleic acid (DNA) that coded for the two different receptors and then ligating, or molecularly stitching them back together, the researchers created DNA coding for chimeric receptors made from combinations of pieces of the receptors with opposite responses to alcohol. This DNA was then introduced into cells that made the corresponding receptor protein, allowing the researchers to examine alcohol's effects on the chimeric receptors. By determining the effects of alcohol on different chimera proteins that contained different combinations of the two receptors, the investigators were able to zero in on which parts of the receptor were important in determining the actions of alcohol. The investigators then compared the amino acid sequence of the receptors in the regions known to confer the differing responses to alcohol. (Proteins are chains whose structure and function are

Figure 1: Defining sites of alcohol action on neurotransmitter receptors



Schematic representations of ligand-gated ion channel type receptors for the neurotransmitters gamma-aminobutyric acid (GABA) and glycine. The function of the $GABA_A$ receptor (rho [ρ] subtype) is inhibited by alcohol, whereas the function of the glycine receptor (alpha [α] subtype) is potentiated by alcohol. Researchers using recombinant deoxyribonucleic acid (DNA) technology created "chimeric" receptors that combined parts of both receptors (Mihic et al. 1997) (white, part derived from $GABA_A$ receptor; black, part derived from glycine receptor). This combination receptor responded to alcohol in a manner that was similar to the response of the glycine receptor; data also suggested that the parts of the receptor that confer alcohol sensitivity appear to reside in part of the receptor within the cell membrane. In addition, the investigators pinpointed specific amino acids within this part of the receptor that played a key role in determining the effect of alcohol. This finding in particular may point toward a specific alcohol interaction site on these receptors.

defined by the sequence of amino acids.) Through these experiments, the investigators were able to pinpoint single-amino acid molecules that conferred a particular response to alcohol within the proteins (Mihic et al. 1997).

Further investigation involved another technique for altering protein structure, site-directed mutagenesis, in which researchers change the DNA coding sequence. This technique is used to make targeted changes in the DNA so that only one amino acid in the protein is altered. The results of these studies indicated that amino acids in the part of the protein that is embedded in the cell membrane are crucial for determining the effect of alcohol on the glycine and $GABA_A$ receptors. This research represents an important step toward identifying the molecular site of action of alcohol at these receptors. Knowing this site of action is expected to be of great help in designing pharmacotherapeutic agents to counteract the effects of alcohol.

Alcohol's Effects on Protein Phosphorylation

In addition to its actions on ligand-gated ion channels, alcohol interacts with other molecules inside neurons. Protein kinases, a class of molecules that have important roles in regulating synaptic transmission and brain function, are enzymes that catalyze the modification of a variety of proteins by promoting the addition of a simple phosphate molecule to specific parts of the proteins (see the section "Setting the Stage: The Structure and Function of Neurons"). This biochemical mechanism, known as protein phosphorylation, is usually activated by intracellular messenger molecules.

Protein phosphorylation can alter the function of a protein by changing the structure of the protein in a subtle way. For example, phosphorylation of some ligand-gated ion channels alters the signaling function of these channels. Thus, it is possible for protein kinase activity to alter

synaptic transmission by altering the function of the receptors involved in transmission. Researchers have long suspected that alcohol exerts some of its effects on brain function by interacting with protein phosphorylation mechanisms.

NMDA-Type Receptors and Fyn Tyrosine Kinase

A recent discovery demonstrates one way in which alcohol's effects on phosphorylation of a ligand-gated ion channel contribute to the intoxicating effects of alcohol and to alcohol tolerance. The NMDA-type glutamate receptor discussed above is altered by phosphorylation of an amino acid known as tyrosine. This alteration takes place through the activity of a protein kinase, known as a protein tyrosine kinase, that is specialized for phosphorylation of the amino acid tyrosine in proteins. The specific kinase involved in the phosphorylation of the NMDA receptors is called Fyn tyrosine kinase.

Mutant mice that lack the gene for Fyn tyrosine kinase (known as Fyn knockout mice) are made using a technique called homologous recombination. This technique involves inserting an altered gene into an embryonic stem cell—a cell that is not differentiated into a specific tissue but has the potential to develop into any type of cell in the organism. The stem cell is then fertilized and implanted into a female mouse to produce embryos. The mice, when mature, have an altered genetic code for the targeted protein. These mice can then breed and establish many generations of animals that have alterations in the expression of a particular protein. The replacement gene in a knockout mouse is nonfunctional; that is, it does not code for an intact, functional protein. Investigators use this experimental approach to examine the roles of many proteins in a variety of cellular and behavioral processes.

Effects of alcohol on Fyn knockout mice have been examined at the level of NMDA receptor molecules, functional synapses, and animal behavior (Miyakawa et al. 1997). Investigators first observed that the Fyn knockout mice were more sensitive to the sedative and movement-incoordinating effects of alcohol than were the matched wild-type control mice (mice without the mutation). In a sleep time test, the animals received an injection of alcohol; the time it took them to get off their backs and stand up was measured. The Fyn knockout mice stayed down longer, indicating that this effect of alcohol lasts longer in the knockout mice than in the control mice.

Because the NMDA receptor has been implicated in the effects of alcohol, the researchers next examined whether alcohol inhibition of synaptic responses mediated by this ligand-gated ion channel was altered in the knockout animals. Interestingly, alcohol inhibited the synaptic response mediated by the NMDA receptor in both wild-type and Fyn knockout mouse synapses. However, the effect in wild-type mice disappeared during the first 20 minutes after alcohol exposure, while the inhibitory effect persisted in the Fyn knockout mice. This loss of alcohol effect within minutes has been called acute tolerance or rapid acute tolerance (Grover et al. 1994; Pearson et al. 1997) and appears to result from molecular changes that counteract the effects of alcohol. Loss of tolerance in the Fyn knockout mice indicates that phosphorylation by Fyn tyrosine kinase is an important step in the loss of alcohol effects on the NMDA receptor.

There are several subtypes of NMDA receptors in the brain. NMDA receptor proteins are complexes composed of a grouping of several individual proteins known as subunits (Anantharam et al. 1992; Ishii et al. 1993; Mishina et al. 1993; Seeburg et al. 1995). The assembly of various receptor subunits confers different functional properties on the receptor. Some evidence indicates that alcohol has more powerful effects on certain NMDA receptor subtypes than on others. For example, alcohol's inhibitory effects on NMDA receptors in neurons appear to be the strongest in receptors that contain a subunit known as NR2B in rats and NRε2 in mice (Fink and Göthert 1996; Lovinger 1995; Yang et al. 1996).

In studies of Fyn knockout mice, treatment with alcohol activates Fyn tyrosine kinase phosphorylation of the NRε2 subunit in wild-type mice; in contrast, phosphorylation of this subunit and the effect of alcohol are absent in the Fyn knockout mice (Miyakawa et al. 1997). Taking together all the findings from the studies of alcohol effects on receptors and the role of Fyn tyrosine kinase, researchers have constructed a scenario in which alcohol produces two important effects on the NMDA receptor. The first effect is inhibition of receptor function, which likely involves an interaction between alcohol and the receptor or an effect of alcohol on the cell membrane at a site very near the receptor. The accumulated evidence for a role of NMDA receptors in acute alcohol intoxication indicates that this inhibitory effect is key in the brain's initial response to alcohol.

The second effect is alcohol activation of Fyn tyrosine kinase, which leads to phosphorylation of the NMDA receptor on the NRε2/2B subunit, counteracting the inhibitory effect of alcohol on the NMDA receptor. The result is a rapid loss of intoxication, even during a single exposure to alcohol. These types of molecular adaptations to alcohol, which lead to behavioral tolerance, appear to alter synaptic communication within the brain. Of note is that Fyn knockout animals have defects in some forms of learning and memory, and they have deficiencies related to long-lasting changes in synaptic transmission that are thought to be involved in learning and memory (Grant et al. 1992). These observations suggest that the NRε2/2B subunit may be involved in the adaptive neuronal processes that store information, including information about alcohol exposure.

How might these molecular effects of alcohol contribute to alcohol abuse and alcoholism? Evidence indicates that sensitivity to alcohol is a predictor of risk for alcoholism. For example, individuals who are able to drink large quantities of alcohol when first exposed to the drug are more likely to keep drinking large quantities and to develop alcohol abuse problems than are persons who can consume only small amounts of alcohol when first drinking.

It is possible that susceptibility to the inhibition of the NMDA receptor by alcohol might differ among individuals, perhaps due to variable expression of NMDA receptor subunits at brain synapses. Individuals with different levels of Fyn tyrosine kinase activity also might differ with respect to the development of rapid tolerance, which could also influence their ability to drink large quantities of alcohol and thus be important in determining susceptibility to alcoholism in different human populations. Although no definitive evidence in humans currently exists to evaluate this hypothesis, the possible role of Fyn tyrosine kinase in determining individual differences in alcohol sensitivity will undoubtedly be explored during the next few years.

Second Messengers and Protein Kinases

Alcohol also may alter the production of intracellular messenger molecules and the distribution of protein kinases in neurons. One way neurotransmitters affect neuronal excitability (other than through receptors that are themselves ion channels) is by acting on receptors that are coupled to intracellular signaling processes. These G protein receptors, so called because they involve the binding and breakdown of guanosine triphosphate (GTP), can influence the activity of the neuron in several ways. For example, neurotransmitter binding to some G protein-coupled receptors leads to the production of small-molecular-weight molecules known as second messengers. One such second-messenger molecule is cyclic adenosine monophosphate (cAMP). The G protein that is activated stimulates an enzyme within cells that leads to rapid production of cAMP, which then interacts with proteins within the cell. The major protein activated by cAMP is known as cAMP-dependent protein kinase. Like the Fyn tyrosine kinase discussed above, this kinase stimulates phosphorylation of proteins within the cell.

The proteins that are phosphorylated upon activation of cAMP-dependent protein kinase include neurotransmitter receptors and neurotransmitter transporters. Phosphorylation of these proteins can lead to changes in protein function, in the distribution of the proteins

within the cell, or both. These changes, in turn, can alter cell function. For example, if either receptor function or the number of receptors at the cell surface changes, the postsynaptic response to a neurotransmitter also changes. If either the actions or the numbers of these neurotransmitter transporters are altered, the duration of neurotransmitter effects within the synapse changes. These types of effects alter communication between neurons and ultimately influence the workings of the entire brain and related animal or human behaviors.

Recent studies indicate that alcohol affects protein kinases in a number of ways. For example, alcohol alters both the amount of protein kinases as well as their intracellular distribution. Protein kinases are found in a number of compartments within cells, but particular kinase molecules are often present within only one part of a cell. This compartmentalization restricts the activity of the kinase so that it can phosphorylate only those proteins in the same part of the cell. This localization, in turn, affects the impact of the kinase on cellular function, because the proteins it phosphorylates have specific functions within that part of the cell. For example, a kinase that is restricted to the nucleus phosphorylates nuclear proteins and most likely affects gene translation. A kinase that is present only in neuronal dendrites may phosphorylate neurotransmitter receptors and alter postsynaptic responses to the neurotransmitter. Thus, the localization of the kinase can determine its impact on the function of an entire neuron.

Scientists recently found that the localization of protein kinases within cells occurs through proteins that attach themselves to the kinases and then anchor the kinases to a particular cellular site (Dell'Aqua and Scott 1997; Mochly-Rosen 1995). Different types of kinases interact with different anchoring proteins. Several different anchoring proteins appear to exist for each kinase, with the various anchoring proteins found in different parts of the cell. Altering the location or number of the anchoring proteins or their ability to interact with the protein kinase appears to change the cellular location of kinases and, hence, the pattern of protein phosphorylation. Such alterations could have important consequences for cellular function.

Long-term exposure to alcohol alters the distribution of cAMP-dependent protein kinase such that a higher concentration of the kinase is found within the cell nucleus during alcohol exposure (Dohrman et al. 1996). Researchers have used a powerful laser confocal microscope to view small parts of neurons and to observe altered distributions of protein kinase. When certain proteins are tagged with fluorescent molecules, they glow when exposed to laser light, making it easy to track these molecules within a cell. Using this technique, researchers have mapped the location of the cAMP-dependent protein kinase within neurons before and after short- and long-term alcohol exposure. The sequestration of the kinase within the cell nucleus following long-term alcohol exposure appears to be the reason for decreased phosphorylation of proteins within the cell cytoplasm and within the membrane enveloping nucleus.

One protein whose function appears to be regulated by cAMP-dependent protein kinase phosphorylation is the transporter for the inhibitory neurotransmitter adenosine. (Neurotransmitter transporters shuttle and help regulate the level of neurotransmitters in the synaptic cleft.) Short-term exposure to alcohol inhibits this transporter molecule in a manner that depends on phosphorylation of the transporter protein or a closely associated protein by the cAMP-dependent protein kinase (Coe et al. 1996). Transporter inhibition increases the extracellular concentration of adenosine because the transporter is no longer able to efficiently remove the neurotransmitter from the synapse. This effect, along with the effects on GABAergic transmission mentioned above, may contribute to the inhibitory effects of alcohol on brain activity.

During chronic alcohol exposure, the sequestering of the kinase in the nucleus leads to reduced transporter phosphorylation, which, in turn, appears to lead to a loss of inhibition of the transporter. Due to this loss, adenosine synapses become more resistant to the effects of alcohol. This resistance translates, over time, to less of an

inhibitory effect with chronic exposure to alcohol. This loss of phosphorylation represents another form of cellular adaptation to alcohol that plays a role in the brain's development of tolerance to and dependence on it.

Long-Term Exposure to Alcohol: Gene Expression, Protein Phosphorylation, and Protein Localization

One way in which alcohol produces a long-lasting change in brain activity is by altering the patterns of expression of proteins that are important for regulating neuronal activity. This regulation often takes place in the nucleus in the form of alterations in gene expression. Messages from the periphery of the cell make their way to the nucleus to interact with DNA and alter gene expression (see the box "From DNA to Protein: How Genetic Information Is Realized" in the previous section).

GABA

Changes in the number or molecular structure of neurotransmitter receptors or other molecules involved in synaptic transmission appear to be involved in the brain's adaptations to the presence of alcohol, which are manifested in tolerance, dependence, and alcohol withdrawal. Two of the neurotransmitter receptors that are sensitive to the acute actions of alcohol are also greatly affected by long-term exposure to alcohol. One of the receptors, $GABA_A$, undergoes structural changes during long-term alcohol exposure. As with the NMDA receptor described above, the $GABA_A$ receptor is a complex of several individual subunit proteins. Different combinations of these subunits can come together to form the receptor channel. One way in which chronic exposure to alcohol affects the $GABA_A$ receptor is to change the expression of different subunit proteins that participate in the formation of the receptor.

The sensitivity of $GABA_A$ receptors to alcohol also appears to be altered as a consequence of long-term exposure to alcohol (Morrow et al. 1988). This loss of alcohol sensitivity may be related to the change in receptor subunit expression (Crews et al. 1996; Mhatre et al. 1993; Morrow 1995). Even when receptor subunit expression is not altered, however, the function of $GABA_A$ receptors is altered by chronic alcohol exposure (Klein et al. 1995). Another mechanism that may contribute to the change in alcohol sensitivity of the $GABA_A$ receptor is a posttranslational modification of the protein—a change that takes place after the last step in gene-directed protein synthesis—such as protein phosphorylation. Research shows that alcohol's effects on the receptor are lost in mice that are genetically engineered to remove the gamma subtype of the phosphorylating enzyme protein kinase C (Harris et al. 1995). Thus, changes in receptor subunit expression and receptor phosphorylation could contribute to alcohol tolerance, because the intoxicating effects of alcohol that involve $GABA_A$ receptors presumably are reduced if these receptors become less sensitive to alcohol.

NMDA

Alterations in the structure or function of NMDA receptor subunit molecules also occur during long-term alcohol exposure. Early reports indicated that the number of NMDA receptors on neurons in brain regions, such as the hippocampus, increased following long-term alcohol exposure (Hoffman 1995). (The hippocampus is a part of the brain thought to play a role in learning and memory as well as in alcohol withdrawal syndrome.) In addition, evidence that NMDA receptor-specific blockers inhibit alcohol withdrawal seizures implicated this receptor in the withdrawal syndrome (Hoffman et al. 1992; Morrisett et al. 1990). Subsequent investigations have provided evidence for increased function of NMDA receptors after chronic alcohol exposure. For example, researchers have examined responses of individual neurons upon exposure to NMDA (a synthetic amino acid that activates the NMDA receptor) by using neurons subjected to long-term alcohol exposure and have compared these responses with those of alcohol-naive neurons. The increases in intracellular calcium produced when the NMDA receptor is activated were enhanced following long-term alcohol exposure (Ahern et al. 1994; Iorio et al. 1992; Smothers et al. 1997).

Alcohol-induced responses of the NMDA receptor appear to be a function of its subunit proteins. The NR1 subunit is present in all receptors and thus seems to be a necessary, or constitutive, element of a functional receptor. The presence or absence of specific NR2 subunits (there are four) is the major factor that underlies variability in the properties of different receptors. Various parts of the brain contain different amounts of each type of NR2 subunit. Certain brain regions contain two of the receptor subtypes and can use either one, or both, as part of the functional NMDA receptor. For example, neurons in the cerebral cortex have receptors that contain both the NR2A and NR2B subunits (Sheng et al. 1994). Changing the amount of either of these subunits changes the functional characteristics of the receptor and alters glutaminergic synaptic transmission within the cerebral cortex.

The increased activity of NMDA receptors after prolonged alcohol exposure most likely arises from increases in the amounts of the particular subunit proteins that contribute to NMDA receptor formation. Brains from animals exposed to alcohol for days to months have been examined to determine whether NMDA receptor subunit expression is altered. Similar experiments have been performed using brain neurons grown in cell culture.

Exposing cultured cerebral cortical neurons to alcohol for a few days increased the amount of ribonucleic acid (RNA) that codes for the NR2B subunit, with little or no change observed in other receptor subunits (Hu et al. 1996). Increased NMDAR2B protein was also observed in these neurons (Follesa and Ticku 1996). This sort of change in the relative amount of each subunit can lead to two consequences with respect to NMDA receptor function. First, the total number of NMDA receptors might increase because a larger amount of subunits is available to construct receptors. This, in turn, could lead to larger synaptic responses at glutaminergic brain synapses. Second, more receptors containing just the NR2B subunit could be expressed, producing a change in the synaptic responses mediated by the receptor. Evidence for increases in relative amounts of receptors containing functional NR2B subunit has been observed following prolonged alcohol exposure (Blevins et al. 1995).

Some research suggests, however, that this long-term, alcohol-induced change in NMDA receptor subunit composition may involve mechanisms other than a simple change in subunit expression (Blevins et al. 1997), as was previously described for the $GABA_A$ receptor (Klein et al. 1995). For example, NMDA receptors containing the NR2B subunit are currently thought to produce longer lasting synaptic responses in the cortex (Carmignoto and Vicini 1992). Responses that last longer will produce a longer lasting excitation of the cell. This extended activity thus may be fundamental to the changes that bring about hyperexcitability of the brain during withdrawal from alcohol.

Alcohol exposure lasting weeks to months leads to increases in the amount of the NR1 and NR2A subunits in several brain regions, including the hippocampus (Snell et al. 1996; Trevisan et al. 1994). The hippocampus plays a key role in learning and memory for certain types of information, and NMDA receptors in this brain region are important to these processes (Kandel et al. 1995). An increase in the amount of the constitutive NR1 subunit of the protein in the hippocampal formation could lead to increased numbers of NMDA receptors in this brain region. Thus, alteration of the number of NMDA receptors is one mechanism that could contribute to the development of memory problems after prolonged alcohol exposure. Increased receptor expression could also contribute to the increased excitability of the brain during withdrawal. Furthermore, excessive activation of NMDA receptors can lead to neuronal injury and death. Thus, increased NMDA receptor expression and function in the hippocampus could contribute to damage in this part of the brain following prolonged alcohol abuse, and this damage could contribute to memory problems. Similar effects are seen in other brain regions, such as the cerebral cortex.

Alcohol Effects on Other Genes

Some genes within neurons appear to be particularly sensitive to alcohol. Several examples of this type of alcohol-sensitive gene have been described in recent studies. For example, neuronal exposure to alcohol leads to increased production of two forms of protein kinase C, an enzyme that mediates protein phosphorylation (Messing et al. 1991). Increased production of these forms results from an increase in the gene-driven production of the RNA that encodes protein kinase C. Thus, researchers suspect that the genes producing these forms of protein kinase C are activated by alcohol. The increased activity of these two forms leads to abnormal growth of neurons (Roivainen et al. 1995). Such improper neuronal growth could contribute to improper brain development as a result of fetal alcohol exposure or improper wiring of neuronal connections, leading to alterations in brain function in adult alcohol abusers. (The chapter on prenatal exposure to alcohol describes in greater detail the impact of alcohol on fetal growth and development.)

In Closing

By altering the function of key proteins—including neurotransmitters and phosphorylating enzymes—within neurons, alcohol can produce changes in synaptic transmission. These rapid changes in the brain result in intoxication. Long-term alcohol exposure produces adaptive changes in the function of these same specialized proteins that lead to alterations in synaptic transmission in a manner that compensates for the lasting presence of alcohol. This adaptation gives rise to tolerance, dependence, and the alcohol withdrawal syndrome. Newly developed techniques for analysis of brain molecules and genetically engineered laboratory animals are allowing researchers to examine the molecular events that are responsible for both the short- and long-term effects of alcohol on the brain. The resulting findings will help scientists understand the effects of alcohol and will also provide the basis for developing pharmaceutical means of diagnosing, treating, and preventing damage from alcohol abuse.

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